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(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
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Stabilised enzyme containing granulates

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Stabilised enzyme containing granulates

Field of the Invention

The present invention relates to the formulation of enzymes, preferably feed-enzymes, into carbohydrate (e.g. starch-) containing granulates, and related to processes for the preparation of such enzyme-containing granulates. These (edible) granulates can then be used in animal feeds and/or human nutrition. The enzyme containing granulates display an improved storage and processing stability.

Background of the Invention

The use of various enzymes in human nutrition or animal, e.g. livestock, feed has become almost common practice. These enzymes are usually produced by culturing micro organisms in large scale fermenters operated by industrial enzyme producers. At the end of the fermentation the resulting "broth" is usually subjected to a series of filtration steps to separate the biomass (the micro organisms) from the desired enzyme (in solution). The enzyme solution is either then sold as a liquid or processed to a dry formulation.

Enzyme liquid and dry formulations are used on a commercial scale by the feed and food industry. Liquid formulations may be added to the feed or food after pelleting in order to avoid heat inactivation of the enzyme(s) which would occur during the pelleting process. However the amounts of enzyme in the final feed or food preparations are usually very small which makes it difficult to achieve a homogenous distribution of the enzyme in the feed or food, and liquids are notoriously more difficult to mix evenly than dry ingredients. In addition one needs specialised (expensive) equipment to add liquids to the feed after pelleting which is not currently available at most feed mills (due to the extra cost).

Dry formulations of enzyme(s), on the other hand, have the disadvantage of heat-inactivation of the enzymes during pelleting. Preferred manufacturing protocols in the feed and food industry involve steam pelleting where the feed or food is subjected to steam injection(s) prior to pelleting. In the subsequent pelleting step the feed or food is forced through a matrix or die and the resulting strips are cut into suitable pellets of variable length. The moisture content immediately before pelleting is generally between 18% and 19%. During this process temperatures may rise to 60-95°C. The combined effect of high moisture content and high temperature is detrimental to most enzymes. These disadvantages are also encountered in other types of thermo mechanical treatments such as extrusion and expansion.

Various enzyme manufacturers have developed alternative formulation methods to try to improve the stability of dry enzyme products during pelleting and storage.

EP 0 758 018 A1 discloses a method to improve the processing and storage stability of dry enzyme preparations by adding inorganic salts.

EP 0 986 313 A1 (WO 98/54980) discloses a process for the preparation of enzyme-containing granulates.

GB 2 167 758 discloses an enzyme granulating method and a granular composition containing an enzyme comprising 1 to 35% by weight of an enzyme and from 0,5 to 30% by weight of a synthetic fibre chip or pulp.

EP 1 069 832 A1 (WO 00/47060) describes a process for the preparation of enzyme containing granulates where an aqueous enzyme-containing liquid is mixed with a solid carrier and optionally additive ingredients and is mechanically processed into granules, dried and subsequently coated with polyethylene glycol.

EP 858 266 (WO 97/16076) discloses a particulate, enzyme-containing preparation suitable for the use in the manufacture of animal feed compositions.

EP 862 623 A1 (WO 97/12958) discloses a microgranular enzyme composition having an average particle size of from about 20 to 400 microns.

None of the cited references discloses an enzyme containing granulate which is stabilized with gummi arabicum or plant proteins.

DE 1 958 104 describes a process for the preparation of enzyme containing granulates suitable for detergent and laundry compositions wherein alkali salts of inorganic or organic acid are used as carrier.

DE 2 137 043 and **DE 2 137 042** disclose an enzyme containing composition suitable for detergent compositions wherein the carrier is comprised of an inorganic salt.

EP 716 685 discloses multiple enzyme granulates suitable for incorporation in detergents and cleaning compositions containing an enzyme and inorganic and/or organic carrier material.

WO 00/36927 discloses a method for producing granulates which contain enzymes and which are suited for feeding animals.

WO 03/059087 describes a process for the preparation of an enzyme-containing granulate wherein an aqueous-containing liquid, optionally supplemented with a solid carrier and/or additive ingredients, is processed into granules, dried and subsequently coated with a polyolefin.

WO 03/059086 describes a process for the preparation of an enzyme-containing granulate wherein an aqueous-containing liquid, optionally supplemented with a solid carrier and/or additive ingredients, is processed into granules, dried and subsequently coated with a dispersion containing particle of a hydrophobic substance, preferably a polyolefin.

Despite the different approaches in the state of the art, there is still an need for stabilized enzyme containing granules that are suitable for human and/or animal nutrition and that display an enhanced stability during processing, pelleting and storage. The granules should be easily obtainable as well as easily compatible with usual food and/or feed ingredients. In addition the granules should be easily processible, e.g. provide low dusting properties, be easy dispersible or mixable in the matrix desired.

Description of the Invention

In a first aspect of the present invention there is provided a process for the preparation of enzyme-containing granule(s), the process comprising processing at least one enzyme, a solid carrier which comprises at least 15% (w/w) of an edible carbohydrate polymer, and at least one stabilizing agent, wherein the stabilizing agent is selected from the group consisting of gummi arabicum and a plant protein and mixtures thereof..

In a second aspect of the present invention there is provided a granule(s) comprising at least one enzyme, a solid carrier which comprises at least 15% (w/w) of an edible carbohydrate polymer, and at least one stabilizing agent, wherein the stabilizing agent is selected from the group consisting of gummi arabicum and plant proteins and mixtures thereof..

In another aspect of the present invention there is provided a granule(s) comprising at least one enzyme, a solid carrier which comprises at least 15% (w/w) of an edible carbohydrate polymer, and at least one stabilizing agent, wherein the stabilizing agent is selected from the group consisting of gummi arabicum and plant proteins and mixtures thereof, wherein the granule is coated.

The terms "granules" or "granule(s)" used throughout the description of the invention, both terms encompassing a single granule as well as a plurality of granules without distinction. The term granules and granulates are used synonymously.

The enzyme-containing granule(s) obtainable by this process seeks to solve or at least mitigate the problems encountered in the prior art. The invention can thus provide processes for the preparation of stabilized enzyme formulations in the form of granulates that use the carbohydrate as a carrier. The carrier may be in particulate or powder form.

A number of prior art documents that refer to pellets containing various enzymes, but these find use as detergents, often in washing compositions. In contrast, the present application finds use in animal feeds or compositions suitable for human nutrition and for that reason the granule(s) of the invention are edible (by animals) and preferably also digestible. It will therefore not be surprising that the granules and compositions of the invention are free of soap, detergents and bleach or bleaching compounds, zeolites, to name but a few.

At least 15% (w/w) of the solid carrier is comprised of an edible carbohydrate polymer (such as starch). Preferably, however, at least 30% (w/w) of the solid carrier comprises the carbohydrate, optimally at least 40% (w/w). Advantageously the major component of the solid carrier is the carbohydrate (e.g. starch), for example more than 50% (w/w), preferably at least 60% (w/w), suitably at least 70% (w/w), and optimally at least 80% (w/w). These weight percentages are based on the total weight of the non-enzymatic components in the final dry granulate.

The edible carbohydrate polymer should be chosen so that it is edible by the animal or human for whom the feed or food, respectively is intended, and preferably digestible as well. The polymer preferably comprises glucose (e.g. a glucose-containing polymer), or $(C_6H_{10}O_5)_n$, units. Preferably the carbohydrate polymer comprises α-D-glucopyranose units, amylose (a linear (1->4) α-D-glucan polymer) and/or amylopectin (a branched D-glucan with α-D-(1->4) and α-D-(1->6) linkages). Starch is the preferred carbohydrate polymer. Other suitable glucose-containing polymers that can be used instead of, or in addition to starch, include α-glucans, β-glucans, pectin (such as proto-pectin), and glycogen. Derivatives of these carbohydrate polymers, such as ethers and/or esters thereof, are also contemplated although gelatinised starch is best avoided and thus may not be present. Suitably the carbohydrate polymer is water-insoluble.

Suitable carbohydrate polymers are corn-, potato- and rice-starch. However, starch obtained from other (e.g. plant, such as vegetable or crop) sources such as tapioca, cassava, wheat, maize, sago, rye, oat, barley, yam, sorghum, or arrowroot is equally applicable. Similarly both native or modified (e.g. dextrin) types of starch can be used in the invention. Preferably the carbohydrate (e.g. starch) contains little or no protein, e.g. less than 5% (w/w), such as less than 2% (w/w) preferably less than 1% (w/w). Regardless of the type of starch (or other carbohydrate polymer) it should be in a form that allows it to be used in an animal feed, in other words an edible or digestible form.

Water may be added to the processing. In a further embodiment of the invention, the granules are dried subsequent to the processing. It is understood that in one embodiment the granules can be dried irrespective of whether water was added to the processing or not.

The enzyme and water are preferably provided as an enzyme-containing (preferably aqueous) liquid, such as a solution or a slurry, which can be mixed with the solid carrier and allowed to absorb onto the carrier. During or after the mixing, the enzyme-containing liquid and the carrier are processed into a granule, which can then subsequently be dried. The use of the carbohydrate carrier may allow the absorption of large amounts of enzyme-containing liquid (and therefore enzyme). The mixture may be used to form a plastic paste or non-elastic dough that can readily be processed into granules, for example it can be extruded.

In the process of the invention the enzyme and water may be present in the same composition before contacting the solid carrier. In this respect, one may provide an enzyme-containing aqueous liquid. This liquid may be a solution or slurry that is from, or derived from, a fermentation process. This fermentation process will usually be one in which the enzyme is produced. The fermentation process may result in a broth that contains the microorganisms (which produce the desired enzyme) and an aqueous solution. This aqueous solution once separated from the micro organisms (for example, by filtration) can be the enzyme-containing aqueous liquid used in the invention. Thus in preferred embodiments the enzyme-containing aqueous liquid is a filtrate, especially a filtrate derived from a fermentation process resulting in production of enzyme. Depending on the process of separation that is used to isolate the enzyme from the fermentation broth, the enzyme can also be present in a retentate.

The amount of enzyme-containing liquid (and so enzyme) that can be absorbed onto the carrier is usually limited by the amount of water that can be absorbed. Preferably the amount of liquid added to the solid carrier is such that (substantially) all the water in the (aqueous) liquid is absorbed by the carbohydrate present in the solid carrier.

At elevated temperatures starch and other carbohydrate polymers can absorb much larger amounts of water under swelling. For this reason the carbohydrate polymer is desirably able to absorb water (or enzyme-containing aqueous liquids). For example, corn starch can absorb up to three times its weight of water at 60°C and up to ten times at 70°C. The use of higher temperatures in order to absorb a greater amount enzyme-containing liquid is thus contemplated by the present invention, and indeed is preferable especially when dealing with thermostable enzymes. For these enzymes therefore the mixing of the solid carrier and liquid (or enzyme and water) and stabilizer can be conducted at elevated temperatures (e.g. above ambient temperature), such as above

30°C, preferably above 40°C and optimally above 50°C. Alternatively or in addition the liquid may be provided at this temperature.

However, in general, non-swelling conditions at lower (e.g. ambient) temperatures are preferred. This may minimise activity loss arising from instability of (heat sensitive) enzymes at higher temperatures. Suitably the temperature during the mixing of the enzyme and water is from 10 to 60°C, such as 10 to 50°C, preferably 20 to 40°C, preferably 20 to 25°C.

The stabilizing agent is selected from the group consisting of gummi arabicum and plant proteins and mixtures thereof. It is understood that the stabilizing agent can be selected from one agent, e.g. only gummi arabicum or be composed of a mixture of e.g. one plant protein and gummi arabicum or a mixture of two or three or more different plant proteins. In a preferred embodiment the stabilizing agent is gummi arabicum. In a preferred embodiment the stabilizing agent is at least one plant protein.

In one embodiment the stabilizing agent is gummi arabicum. Other words for gummi arabicum are gum Arabic or gum acacia. Gummi arabicum is prepared from an exudate from the stems and branches Acadia trees (Leguminosae), e.g. Acadia trees of sub-Saharan (Sahel zone) Acacia Senegal and Acacis seyal trees and produced naturally as large nodules during a process called gummosis to seal wounds in the bark of the tree. Gummi arabicum is is a complex and variable mixture of arabinogalactan oligosaccharides, polysaccharides and glycoproteins. Depending on the source, the glycan components contain a greater proportion of L-arabinose relative to D-galactose (Acaia seyal) or D-galactose relative to L-arabinose (Acacia senegal). The gum from Acacia *seyal* also contains significantly more 4-O-methyl-D-glucuronic acid but less L-rhamnose and unsubstituted D-glucuronic acid than that from Acaia *senegal.* Gummi arabicum according to the invention encompasses Gummi arabicum from Acacia Senegal and/or from Acacia seyal and/or from Acacia laeta and/or from Acacia karrod.

Gummi arabicum is a mixture of branched glycoproteins with major part of ß-1,3-galactopyran core with rhamnoglucuronoarabinogalactose pentasaccharide side chains. In a preferred embodiment gummi arabicum with a molecular weight M_R of 100 000 to 2 000 000, preferably 200 000 to 1 500 000, preferably 300 000 to 1 200 000 is used.

Gummi arabicum is commercially available under the CAS No 9000-01-5, for example from Colloides Naturels International CNI (129 Chemin de Croisset, PO BOX 4151 - 76723 Rouen Cédex, France).

Gummi arabicum is registered as technological additive for food under the classification E 414 (Reference: Merck Index, 12, 11. SAX, AQQ 500).

In one embodiment the stabilizing agent is at least one plant protein. A plant protein according to the invention can be obtained from any plant, e.g. from grains (cereal), pulses, vegetables or fruits. The plant protein can be isolated from any kind of the plant, such as leaf, seed, stem, flower etc. As a plant protein will always be isolated from a plant, it can contain up to 20% (by weight), of non- protein components preferably up to 15%, preferably up to 10, especially up to 5%. A plant protein according to the invention can also be a complete or partial hydrolysate obtained from a plant protein.

A plant protein can for example be obtained from a grain (cereal) such as wheat, rye, oats, barley, corn (zea oryza), rice and millet The protein fractions of these grains can be used as single fractions or as mixtures. Protein fractions of grains are albumins, globulins, prolamins and Glutelins, the so called Osborne-Fractions. The following table list the protein fraction of the preferred grains

Fraction	wheat	Rye	oats	barley	corn	rice	Millet
Albumine	Leucosin						
Globulina	Edestin		Avenalin				
Prolamine	Gliadin	Secalin	Gliadin	Hordein	Zein	Oryzin	kafirin
Gluteline	glutenin	secalinin	avenin	hordenin	zeanin	oryzenin	

In one embodiment of the invention the single fraction of a grain can be used or a protein, which consists of a mixture of the above mentioned fractions.

In a preferred embodiment a grain protein (=cereal protein) is used, which is obtained from wheat, especially the so-called wheat Gluten, which encompasses the prolamin und glutelin fractions of wheat

A plant protein can be obtained from beans and peas (pulses, Legumes), including but not limited to beans and peas from the family of the Papilionaceae, e.g. such as as soy beans (Glycine max), Vicia faba, green beans (Phaseolus vulgaris, P. coccineus), lima beans (Phaseolus lunatus), green peas (Pisa sative) as well as peanuts (Arachis hyopgaea) and cashews.

In a preferred embodiment the plant protein is selected from grain proteins and/or pulses proteins.

A plant protein can be obtained from vegetables including but not limited to potatoes, sweet potatoes, tomatoes, cucumbers, sprouts, cabbage etc.

A plant protein can be obtained from fruits including but not limited to bananas, apples, pears etc.

The following table list commercially available plant proteins, which can be used according to the invention

Plant protein	name		batch	Producer/distributor	CAS-No..	Other numbers
Soyprotein	Supro 670	IP Non GM	IP 094/2003	Protein Technologies International	9010-10-0	
Soyprotein	Supro 1751	GMO frei	M 330000575	Protein Technologies International	9010-10-0	EG-Nr. 200-061-5
potato protein	Alburex	SP		Roquette	100209-45-8	EINECS 309-353-8
wheat protein	Solpro 100		E 031579	Amylum	RN 100684-25-1	

In a preferred embodiment the plant protein is selected from a plant of the family of Fabaceae, Poaceae, Leguminosae and Solanaceae and mixtures thereof.

In a preferred embodiment the plant protein is selected from the group consisting of soy protein, wheat protein and potato protein and mixtures thereof.

The plant protein used as stabilizers according to the invention can for example be obtained from the following plants.

Anacardiaceae such as the genera Pistacia, Mangifera, Anacardium e.g. the species *Pistacia vera* [pistachios], *Mangifer indica* [Mango] or *Anacardium occidentale* [Cashew].

Asteraceae such as the genera Calendula, Carthamus, Centaurea, Cichorium, Cynara, Helianthus, Lactuca, Locusta, Tagetes, Valeriana e.g. the species Calendula officinalis [Marigold], Carthamus tinctorius [safflower], *Centaurea cyanus* [cornflower], *Cichorium intybus* [blue daisy], Cynara scolymus [artichoke], *Helianthus annus* [sunflower], *Lactuca sativa, Lactuca crispa, Lactuca esculenta, Lactuca scariola L. ssp. sativa, Lactuca scariola L. var. integrata, Lactuca scariola L. var. integrifolia, Lactuca sativa subsp. romana, Locusta communis, Valeriana locusta* [lettuce], *Tagetes lucida, Tagetes erecta* or *Tagetes tenuifolia* [marigold].

Apiaceae such as the genera Daucus e.g. the species *Daucus carota* [carrot]

Betulaceae such as the genera Corylus (Hazel) e.g. the species *Corylus avellana* or *Corylus colurna* [hazelnut],

Boraginaceae such as the genera Borago e.g. the species *Borago officinalis* [borage]

Brassicaceae such as the genera Brassica, Melanosinapis, Sinapis, Arabadopsis e.g. the species *Brassica napus, Brassica rapa* ssp. [canola, oilseed rape, turnip rape], *Sinapis arvensis Brassica juncea, Brassica juncea var. juncea, Brassica juncea var. crispifolia, Brassica juncea var. foliosa, Brassica nigra, Brassica sinapioides, Melanosinapis communis* [mustard], *Brassica oleracea* [fodder beet] or Arabidopsis thaliana.

Bromeliaceae such as the genera Anana, Bromelia (pineaple) e.g. the species *Anana comosus, Ananas ananas* or *Bromelia comosa* [pineapple].

Caricaceae such as the genera Carica e.g. the species *Carica papaya* [papaya]

Cannabaceae such as the genera Cannabis e.g. the species *Cannabis sative* [hemp]

Convolvulaceae such as the genera Ipomea, Convolvulus e.g. the species *Ipomoea batatus, Ipomoea pandurata, Convolvulus batatas, Convolvulus tiliaceus, Ipomoea fastigiata, Ipomoea tiliacea, Ipomoea triloba* or *Convolvulus panduratus* [Batate, sweet potato, Man of the Earth, wild potato]

Chenopodiaceae such as the genera Beta, i.e. the species *Beta vulgaris, Beta vulgaris var. altissima, Beta vulgaris var. Vulgaris, Beta maritima, Beta vulgaris var. perennis, Beta vulgaris var. conditiva* or *Beta vulgaris var. esculenta* [sugar beet].

Cucurbitaceae such as the genera Cucubita e.g. the species *Cucurbita maxima, Cucurbita mixta, Cucurbita pepo* or *Cucurbita moschata* [pumpkin, squash]

Elaeagnaceae such as the genera Elaeagnus e.g. the species *Olea europaea* [olive]

Ericaceae such as the genera Kalmia e.g. the species *Kalmia latifolia, Kalmia angustifolia, Kalmia microphylla, Kalmia polifolia, Kalmia occidentalis, Cistus chamaerhodendros* or *Kalmia lucida* [American laurel, broad-leafed laurel, calico bush, spoon wood, sheep laurel, alpine laurel, bog laurel, western bog-laurel, swamp-laurel]

Euphorbiaceae such as the genera Manihot, Janipha, Jatropha, Ricinus e.g. the species *Manihot utilissima, Janipha manihot,, Jatropha manihot., Manihot aipil, Manihot dulcis, Manihot manihot, Manihot melanobasis, Manihot esculenta* [manihot, arrowroot,

tapioca, cassava] or *Ricinus communis* [castor bean, Castor Oil Bush, Castor Oil Plant, Palma Christi, Wonder Tree]

Fabaceae such as the genera Pisum, Albizia, Cathormion, Feuillea, Inga, Pithecolobium, Acacia, Mimosa, Medicajo, Glycine, Dolichos, Phaseolus, Soja e.g. the species *Pisum sativum, Pisum arvense, Pisum humile* [pea], *Albizia berteriana, Albizia julibrissin, Albizia lebbeck, Acacia berteriana, Acacia littoralis, Albizia berteriana, Albizzia berteriana, Cathormion berteriana, Feuillea berteriana, Inga fragrans, Pithecellobium berterianum, Pithecellobium fragrans, Pithecolobium berterianum, Pseudalbizzia berteriana, Acacia julibrissin, Acacia nemu, Albizia nemu, Feuilleea julibrissin, Mimosa julibrissin, Mimosa speciosa, Sericanrda julibrissin, Acacia lebbeck, Acacia macrophylla, Albizia lebbek, Feuilleea lebbeck, Mimosa lebbeck, Mimosa speciosa* [bastard logwood, silk tree, East Indian Walnut], *Medicago sativa, Medicago falcata, Medicago varia* [alfalfa] *Glycine max Dolichos soja, Glycine gracilis, Glycine hispida, Phaseolus max, Soja hispida* or *Soja max* [soybean]

Geraniaceae such as the genera Pelargonium, Cocos, Oleum e.g. the species *Cocos nucifera, Pelargonium grossularioides* or *Oleum cocois* [coconut]

Gramineae such as the genera Saccharum e.g. the species Saccharum officinarum

Juglandaceae such as the genera Juglans, Wallia e.g. the species *Juglans regia, Juglans ailanthifolia, Juglans sieboldiana, Juglans cinerea, Wallia cinerea, Juglans bixbyi, Juglans californica, Juglans hindsii, Juglans intermedia, Juglans jamaicensis, Juglans major, Juglans microcarpa, Juglans nigra* or *Wallia nigra* [walnut, black walnut, common walnut, persian walnut, white walnut, butternut, black walnut]

Lauraceae such as the genera Persea (avocado), Laurus e.g. the species laurel *Laurus nobilis*, [bay, laurel, bay laurel, sweet bay], *Persea americana, Persea gratissima* or *Persea persea* [avocado]

Leguminosae such as the genera Arachis e.g. the species *Arachis hypogaea* [peanut]

Linaceae such as the genera Linum, Adenolinum e.g. the species *Linum usitatissimum, Linum humile, Linum austriacum, Linum bienne, Linum angustifolium, Linum catharticum, Linum flavum, Linum grandiflorum, Adenolinum grandiflorum, Linum lewisii, Linum narbonense, Linum perenne, Linum perenne var. lewisii, Linum pratense* or *Linum trigynum* [flax, linseed]

Lythrarieae such as the genera Punica e.g. the species *Punica granatum* [Pomegranate]

Malvaceae such as the genera Gossypium e.g. the species *Gossypium hirsutum, Gossypium arboreum, Gossypium barbadense, Gossypium herbaceum* or *Gossypium thurberi*, [cotton]

Musaceae such as the genera Musa e.g. the species *Musa nana, Musa acuminata, Musa paradisiaca, Musa* spp. [banana]

Onagraceae such as the genera Camissonia, Oenothera e.g. the species *Oenothera biennis* or *Camissonia brevipes* [primrose, evening primrose]

Palmae such as the genera Elacis e.g. the species *Elaeis guineensis* [oil plam]

Papaveraceae such as the genera Papaver e.g. the species *Papaver orientale, Papaver rhoeas, Papaver dubium* [poppy, oriental poppy, corn poppy, field poppy, shirley poppies, field poppy, long-headed poppy, long-pod poppy]

Pedaliaceae such as the genera Sesamum e.g. the species *Sesamum indicum* [sesame]

Piperaceae such as the genera Piper, Artanthe, Peperomia, Steffensia e.g. the species *Piper aduncum, Piper amalago, Piper angustifolium, Piper auritum, Piper betel, Piper cubeba, Piper longum, Piper nigrum, Piper retrofractum, Artanthe adunca, Artanthe elongata, Peperomia elongata, Piper elongatum, Steffensia elongata*. [Cayenne pepper, wild pepper]

Poaceae such as the genera Hordeum, Secale, Avena, Sorghum, Andropogon, Holcus, Panicum, Oryza, Zea (corn), Triticum e.g. the species *Hordeum vulgare, Hordeum jubatum, Hordeum murinum, Hordeum secalinum, Hordeum distichon Hordeum aegiceras, Hordeum hexastichon., Hordeum hexastichum, Hordeum irregulare, Hordeum sativum, Hordeum secalinum* [barley, pearl barley, foxtail barley, wall barley, meadow barley], *Secale cereale* [rye], Avena sativa, *Avena fatua, Avena byzantina, Avena fatua var. sativa, Avena hybrida* [oat], *Sorghum bicolor, Sorghum halepense, Sorghum saccharatum, Sorghum vulgare, Andropogon drummondii, Holcus bicolor, Holcus sorghum, Sorghum aethiopicum, Sorghum arundinaceum, Sorghum caffrorum, Sorghum cernuum, Sorghum dochna, Sorghum drummondii, Sorghum durra, Sorghum guineense, Sorghum lanceolatum, Sorghum nervosum, Sorghum saccharatum, Sorghum subglabrescens, Sorghum verticilliflorum, Sorghum vulgare, Holcus halepensis, Sorghum miliaceum* millet, *Panicum militaceum* [Sorghum, millet], *Oryza sativa, Oryza latifolia* [rice], *Zea mays* [corn, maize] *Triticum aestivum, Triticum durum, Triticum turgidum, Triticum hybernum, Triticum macha, Triticum sativum* or *Triticum vulgare* [wheat, bread wheat, common wheat]

Proteaceae such as the genera Macadamia e.g. the species *Macadamia intergrifolia* [macadamia]

Rubiaceae such as the genera Coffea e.g. the species Cofea spp., *Coffea arabica*, *Coffea canephora* or *Coffea liberica* [coffee]

Scrophulariaceae such as the genera Verbascum e.g. the species *Verbascum blattaria*, *Verbascum chaixii*, *Verbascum densiflorum*, *Verbascum lagurus*, *Verbascum longifolium*, *Verbascum lychnitis*, *Verbascum nigrum*, *Verbascum olympicum*, *Verbascum phlomoides*, *Verbascum phoenicum*, *Verbascum pulverulentum* or *Verbascum thapsus* [mullein, white moth mullein, nettle-leaved mullein, dense-flowered mullein, silver mullein, long-leaved mullein, white mullein, dark mullein, greek mullein, orange mullein, purple mullein, hoary mullein, great mullein]

Solanaceae such as the genera Capsicum, Nicotiana, Solanum, Lycopersicon e.g. the species *Capsicum annuum*, *Capsicum annuum var. glabriusculum*, *Capsicum frutescens* [pepper], *Capsicum annuum* [paprika], *Nicotiana tabacum*, *Nicotiana alata*, *Nicotiana attenuata*, *Nicotiana glauca*, *Nicotiana langsdorffii*, *Nicotiana obtusifolia*, *Nicotiana quadrivalvis*, *Nicotiana repanda*, *Nicotiana rustica*, *Nicotiana sylvestris* [tobacco], *Solanum tuberosum* [potato], *Solanum melongena* [egg-plant] (*Lycopersicon esculentum*, *Lycopersicon lycopersicum.*, *Lycopersicon pyriforme*, *Solanum integrifolium* or *Solanum lycopersicum* [tomato]

Sterculiaceae such as the genera Theobroma e.g. the species *Theobroma cacao* [cacao]

Theaceae such as the genera Camellia e.g. the species *Camellia sinensis*) [tea]

The stabilizing agent in usually added in an amount of 0.01 to 30 %, such as 1 to 20%, such as 3 to 10 weight-% based on the total weight of the mixture to be processed. The stabilizing agent(s) can either be mixed with the carrier or with the enzyme.

The mechanical processing used in the present invention for making the mixture of the enzyme, optionally water (e.g. an enzyme-containing liquid), the stabilizing agent and the solid carrier into granules (in other words granulating) can employ known techniques frequently used in food, feed and enzyme formulation processes. This may comprise expansion, extrusion, spheronisation, pelleting, high shear granulation, drum granulation, fluid bed agglomeration or a combination thereof. These processes are usually characterised by an input of mechanical energy, such as the drive of a screw, the rotation of a mixing mechanism, the pressure of a rolling mechanism of a pelleting apparatus, the movement of particles by a rotating bottom plate of a fluid bed agglo-

merator or the movement of the particles by a gas stream, or a combination thereof. These processes allow the solid carrier (e.g. in the form of a powder), to be mixed with the enzyme and optionally water, for example an enzyme-containing liquid (an aqueous solution or slurry), the stabilizer, and so subsequently granulated.

Alternatively the solid carrier can be mixed with the enzyme (e.g. in a powder form) and the stabilizing agent, to which optionally water, such as a liquid (or slurry) can then be added (which can act as granulating liquid).

In yet a further embodiment of the invention the granules (e.g. an agglomerate) is formed by spraying or coating the enzyme-containing liquid onto the carrier, which was previously mixed with the stabilizing agent, such as in a fluid bed agglomerator. Here the resulting granules can include an agglomerate as can be produced in a fluid bed agglomerator.

Preferably the mixing of the enzyme-containing liquid, the solid carrier and the stabilizing agent additionally comprises kneading of the mixture. This may improve the plasticity of the mixture in order to facilitate granulation (e.g. extrusion).

In a preferred embodiment the granulate is formed by extrusion, preferably by extrusion at low pressure. This may offer the advantage that the temperature of the mixture being extruded will not, or only slightly, increase. Low-pressure extrusion includes extrusion for example in a Fuji Paudal basket- or dome- extruder. The extrusion may naturally produce granules (the granules may break off after passage through a die) or a cutter may be employed.

Suitably the granules will have a water content of from 15 to 50%, such as 20 to 40%, such as from 25 to 35, preferably 33 to 37% prior to drying. The enzyme content of the granules is preferably from 1 to 25%, such as 3 to 15, such as 5 to 12% (e.g. at least 50,000 ppm) prior to drying. (always calculated as weight % based on the total weight of the granule).

The granules obtained can be subjected to rounding off (e.g. spheronisation), such as in a spheromiser, e.g. a MARUMERISER™ machine and/or compaction. If the obtained granules are dried, the spheronisation is preferably conducted prior to drying. The granules can be spheronised prior to drying since this may reduce dust formation in the final granulate and/or may facilitate any coating of the granulate.

The granules can then be dried, such as in a fluid bed drier or, in case of the fluid bed agglomeration, can be immediately dried (in the agglomerator) to obtain (solid dry) granules. Other known methods for drying granules in the food, feed or enzyme industry can be used by the skilled person. Suitably the granulate is flowable. The drying

preferably takes place at a temperature of from 25 to 60°C, such as 30 to 50°C. Here the drying may last from 10 minutes to several hours. The length of time required will of course depend on the amount of granules to be dried.

After drying the granules, the resulting dried granules preferably have a water content of from 3 to 10%, such as from 5 to 9% by weight.

In a preferred embodiment of the invention there is provided a process wherein the process comprises:

a) mixing an aqueous liquid containing the enzyme with the solid carrier and the stabilizing agent;
b) mechanically processing the mixture obtained in a) to obtain enzyme-containing granules; and
c) drying the enzyme-containing granule(s) obtained in b).

In a further embodiment of the invention the granules are coated. A coating may be applied to the granule to give additional (e.g. favoured) characteristics or properties, like low dust content, colour, protection of the enzyme from the surrounding environment, different enzyme activities in one granulate or a combination thereof. The granules can be coated with or without prior drying. The granules can be coated with a fat, wax, polymer, salt, unguent and/or ointment or a coating (e.g. liquid) containing a (second) enzyme or a combination thereof. It will be apparent that if desired several layers of (different) coatings can be applied. To apply the coating(s) onto the granulates a number of known methods are available which include the use of a fluidised bed, a high shear granulator, a mixer granulator, or a Nauta-mixer.

In one embodiment the granules are coated, preferably after drying, for example to a residual moisture of less than about 10% by weight, with an organic polymer which is suitable for feedstuffs, by

(a) spraying the granules in a fluidized bed with a melt, a solution or a dispersion of the organic polymer or carrying out in a fluidized bed a powder coating with the organic polymer; or
(b) coating the granules in a mixer by melting on the organic polymer, or spraying the crude granulate with a melt, a solution or a dispersion of the organic polymer or carrying out a powder coating with the organic polymer;

and if necessary post-drying, cooling and/or freeing from coarse fractions the respective resultant polymer-coated granules.

According to a preferred embodiment of the process of the invention, the granules are charged into a fluidized bed, fluidized and coated with an aqueous or non-aqueous, preferably aqueous, solution or dispersion of the organic polymer by spraying. For this purpose a liquid which is as highly concentrated as possible and still sprayable is used, for example a from 10 to 50% strength by weight aqueous or non-aqueous solution or dispersion of at least one polymer which is selected from the group consisting of

a) polyalkylene glycols, in particular polyethylene glycols having a number average molecular weight of from about 400 to 15,000, for example from about 400 to 10,000;
b) polyalkylene oxide polymers or copolymers having a number average molecular weight of from about 4000 to 20,000, for example from about 7700 to 14,600; in particular block copolymers of polyoxyethylene and polyoxypropylene;
c) polyvinylpyrrolidone having a number average molecular weight from about 7000 to 1,000,000, for example from about 44,000 to 54,000
d) vinylpyrrolidone/vinylacetate copolymers having a number average molecular weight from about 30,000 to 100,000, for example from about 45,000 to 70,000;
e) polyvinyl alcohol having a number average molecular weight from about 10,000 to 200,000, for example from about 20,000 to 100,000; and
f) hydroxypropyl methyl cellulose having a number average molecular weight from about 6000 to 80,000, for example from about 12,000 to 65,000.

According to a further preferred process variant, for the coating a from 10 to 40% strength by weight, preferably from about 20 to 35% strength by weight, sprayable aqueous or non-aqueous solution or dispersion of at least one polymer which is selected from the group consisting of:

g) alkyl (meth)acrylate polymers and copolymers having a number average molecular weight from about 100,000 to 1,000,000; in particular ethyl acrylate/methyl methacrylate copolymers and methyl acrylate/ethyl acrylate copolymers; and
h) polyvinyl acetate having a number average molecular weight from about 250,000 to 700,000, possibly stabilized with polyvinylpyrrolidone is used.

Generally, preference is given to aqueous solutions or aqueous dispersions for the following reasons: No special measures are necessary for working up or recovering the solvents; no special measures are required for explosion protection; some coating materials are preferentially offered as aqueous solutions or dispersions.

However, in special cases, the use of a non-aqueous solution or dispersion can also be advantageous. The coating material dissolves very readily or an advantageously high proportion of the coating material can be dispersed. In this manner a spray liquid having a high solids content can be sprayed, which leads to shorter process times. The

lower enthalpy of evaporation of the non-aqueous solvent also leads to shorter process times.

Dispersions which can be used according to the invention are obtained by dispersing above polymers in an aqueous or non-aqueous, preferably aqueous, liquid phase, with or without a customary dispersant. A polymer solution or dispersion is preferably sprayed in such a manner that the granules are charged into a fluidized-bed apparatus or a mixer and the spray material is sprayed on with simultaneous heating of the charge. The energy is supplied in the fluidized-bed apparatus by contact with heated drying gas, frequently air, and in the mixer by contact with the heated wall and, if appropriate, with heated mixing tools. It may be expedient to preheat the solution or dispersion if as a result spray material can be sprayed with a high dry matter content. When organic liquid phases are used, solvent recovery is expedient. The product temperature during the coating should be in the range of from about 35 to 50°C. The coating can be carried out in the fluidized-bed apparatus in principle in the bottom-spray process (nozzle is in the gas-distributor plate and sprays upwards) or in the top-spray process (coating is sprayed from the top into the fluidized bed).

Examples of suitable polyalkylene glycols a) are: polypropylene glycols, and in particular polyethylene glycols of varying molar mass, for example PEG 4000 or PEG 6000, obtainable from BASF AG under the tradenames Lutrol E 4000 and Lutrol E 6000.

Examples of above polymers b) are: polyethylene oxides and polypropylene oxides, ethylene oxides/propylene oxide mixed polymers and block copolymers made up of polyethylene oxide and polypropylene oxide blocks, for example polymers which are obtainable from BASF AG under the tradenames Lutrol F 68 and Lutrol F127.
Of the polymers a) and b), preferably, highly concentrated solutions of from up to about 50% by weight, for example from about 30 to 50% by weight, based on the total weight of the solution, can advantageously be used.

Examples of above polymers c) are: polyvinylpyrrolidones, as are marketed, for example, by BASF AG under the tradenames Kollidon or Luviskol. Of these polymers, highly concentrated solutions having a solids content of from about 30 to 40% by weight, based on the total weight of the solution, can advantageously be used.

An example of abovementioned polymers d) is a vinylpyrrolidone/vinyl acetate copolymer which is marketed by BASF AG under the tradename Kollidon VA64. Highly concentrated solutions of from about 30 to 40% by weight, based on the total weight of the solution, of these copolymers can particularly advantageously be used.

Examples of above polymers e) are: products such as are marketed, for example, by Hoechst under the tradename Mowiol. Solutions of these polymers having a solids content in the range from about 8 to 20% by weight can advantageously be used.

Examples of suitable polymers f) are: hydroxypropylmethyl-celluloses, for example as marketed by Shin Etsu under the tradename Pharmacoat.

Examples of abovementioned polymers g) are: alkyl (meth)acrylate polymers and copolymers whose alkyl group has from 1 to 4 carbon atoms. Specific examples of suitable copolymers are: ethyl acrylate/methyl methacrylate copolymers, which are marketed, for example, under the tradenames Kollicoat EMM 30D by BASF AG or under the tradenames Eutragit NE 30 D by Röhm; also methacrylate/ethyl acrylate copolymers, as are marketed, for example, under the tradenames Kollicoat MAE 30DP by BASF AG or under the tradenames Eutragit 30/55 by Röhm. Copolymers of this type can be processed according to the invention, for example, as from 10 to 40% strength by weight dispersions.

Examples of above polymers h) are: polyvinyl acetate dispersions which are stabilized with polyvinylpyrrolidone and are marketed, for example, under the tradename Kollicoat SR 30D by BASF AG (solids content of the dispersion from about 20 to 30% by weight).

According to a further preferred embodiment of the process of the invention, the granules are charged into a fluidized bed and powder-coated. The powder-coating is preferably carried out using a powder of a solid polymer which is selected from the group consisting of hydroxypropyl methyl celluloses (HPMC) having a number average molecular weight of from about 6000 to 80,000; in a mixture with a plasticizer. Suitable materials for a powder coating are also all other coating materials which can be present in the pulverulent form and can be applied neither as a melt nor as highly concentrated solution (for example the case with HPMC).

The powder coating is preferably carried out in such a manner that the coating material is continuously added to the granules charged into the fluidized bed. The fine particles of the coating material (particle size in the range of from about 10 to 100 µm) lie on the relatively rough surface of the crude granulate. By spraying in a plasticizer solution, the coating material particles are stuck together. Examples of suitable plasticizers are polyethylene glycol solutions, triethyl citrate, sorbitol solutions, paraffin oil and the like. To remove the solvent, the coating is performed with slight heating. The product temperature in this case is below about 60°C, for example from about 40 to 50°C.
In principle, the powder coating can also be carried out in a mixer. In this case, the powder mixture is added and the plasticizer is also injected via a nozzle. Drying is performed by supplying energy via the wall of the mixer and if appropriate via the mixing

tools. Here also, as in the coating and drying in the fluidized bed, low product temperatures must be maintained.

According to a further preferred embodiment of the process of the invention, the granules are charged into a fluidized bed or mixer are coated using a melt of at least one polymer which is selected from the group consisting of

a) polyalkylene glycols, in particular polyethylene glycols, having a number average molecular weight of from about 1000 to 15,000; and

b) polyalkylene oxide polymers or copolymers having a number average molecular weight of from about 4000 to 20,000, in particular block copolymers of polyoxyethylene and polyoxypropylene.

The melt coating is carried out in a fluidized bed preferably in such a manner that the granulate to be coated is charged into the fluidized-bed apparatus. The coating material is melted in an external reservoir and pumped to the spray nozzle, for example, via a heatable line. Heating the nozzle gas is expedient. Spraying rate and melt inlet temperature must be set in such a manner that the coating material still runs readily on the surface of the granulate and coats this evenly. It is possible to preheat the granulate before the melts are sprayed. In the case of coating materials having a high melting point, attention must be paid to the fact that the product temperature must not be set too high in order to minimize loss of enzyme activity. The product temperature should be in the range of from about 35 to 50°C. The melt coating can also be carried out in principle by the bottom-spray process or by the top-spray process. The melt coating can be carried out in a mixer in two different ways. Either the granulate to be coated is charged into a suitable mixer and a melt of the coating material is sprayed into the mixer, or, in another possibility, the coating material in solid form is to be mixed with the product. By supplying energy via the vessel wall or via the mixing tools, the coating material is melted and thus coats the crude granulate. If required, some release agent can be added from time to time. Suitable release agents are, for example, salicic acid, talcum, stearates and tricalcium phosphate.

The polymer solution, polymer dispersion or polymer melt used for the coating may receive other additions, for example of microcrystalline cellulose, talcum or kaolin.

In another embodiment of the invention the granules can be coated with a polyolefin as described in WO 03/059087, page 2, lines 19 to page 4, line 15.

In another embodiment of the invention the granules can be coated with a dispersion comprising particle of a hydrophobic substance dispersed in a suitable solvent as described in WO 03/059086, page 2, line 18 to page 4 line 8. In a preferred embodiment

of this coating, a polyolefin, especially preferred polyethylene and/or polypropylen are used.

In other embodiments additional ingredients can be incorporated into the granulate e.g. as processing aids, for further improvement of the pelleting stability and/or the storage stability of the granulate. A number of such preferred additives are discussed below. Salts may be included in the granulate, (e.g. with the solid carrier or water). Preferably (as suggested in EP-A-0,758,018) inorganic salt(s) can be added, which may improve the processing and storage stability of the dry enzyme preparation. Preferred inorganic salts are water soluble. They may comprise a divalent cation, such as zinc (in particular), magnesium, and calcium. Sulphate is the most favoured anion although other anions resulting in water solubility can be used. The salts may be added (e.g. to the mixture) in solid form. However, the salt(s) can be dissolved in the water or enzyme-containing liquid prior to mixing with the solid carrier. Suitably the salt is provided at an amount that is at least 15% (w/w based on the enzyme), such as at least 30%. However, it can be as high as at least 60% or even 70% (again, w/w based on the enzyme). These amounts can apply to the granules either before or after drying. The granules may therefore comprise less than 12% (w/w) of the salt, for example from 2.5 to 7.5%, e.g. from 4 to 6%. If the salt is provided in the water then it can be in an amount of from 5 to 30% (w/w), such as 15 to 25%.

Further improvement of the pelleting stability may be obtained by the incorporation of hydrophobic, gel-forming or slow dissolving (e.g. in water) compounds. These may be provided at from 1 to 10%, such as 2 to 8%, and preferably from 4 to 6% by weight (based on the weight of water and solid carrier ingredients). Suitable substances include derivatised celluloses, such as HPMC (hydroxy-propyl-methyl-cellulose), CMC (carboxy-methyl-cellulose), HEC (hydroxy-ethyl-cellulose); polyvinyl alcohols (PVA); and/or edible oils. Edible oils, such as soy oil or canola oil, can be added (e.g. to the mixture to be granulated) as a processing aid.

Preferably the granules have a relatively narrow size distribution (e.g. they are monodisperse). This can facilitate a homogeneous distribution of the enzyme in the granules in the animal feed. The process of the invention tends to produce granulates with a narrow size distribution. However, if necessary, an additional step can be included in the process to further narrow the size distribution of the granules, such as screening. The mean particle size distribution of the granulate is suitably between 100 µm and 2000 µm, preferably between 200 µm and 1800 µm, preferably between 300 µm and 1600 µm. The granules may be of irregular (but preferably regular) shape, for example approximately spherical. In a preferred embodiment the granules have a mean particle size distribution between 500 and 2000 µm, preferably between 500 and 1800µm, preferably between 600 and 1000 µm. The mean particle size distribution is determined by using Mastersizer S, a machine of Malvern Instruments GmbH, Serial No., 32734-

08. The mean particle size distribution is characterized by the values of D(v,0.1), D(v,0.5) and D(v,0.9) as well as the mean particle size of the distribution D(4,3).

The water or enzyme-containing liquid may comprise one or more enzyme(s) and are usually of microbial origin, e.g. obtained from a microbial fermentation. Usually the enzyme will be in an active form (for example it may have catalytic or physiological activity). Preferably the liquid is in a concentrated form, such as an ultra-filtrate (UF), which may allow the production of a granulate with a desired activity level.

Preferred enzymes for the granules of the present invention include those enzymes useful in food (including baking) and feed industries. Such enzymes include but are not limited to proteases (bacterial, fungal, acid, neutral or alkaline), preferably with a neutral and/or acidic pH optimum, amylases (alpha or beta), lipases (fungal, bacterial, mammalian), preferably phospholipases such as the mammalian pancreatic phospholipases A2, cellulase (whole cellulase or functional components thereof,), xylanases, galactanases; peptidases, galactosidases, pectinases, esterases, phosphatases, such as phytases (both 3-phytases and 6-phytases) and/or acid phosphatases and glucose oxidases and mixtures thereof.

Suitable enzyme(s) are those to be included in animal feed which includes pet food and/or in human nutrition. The function of these enzymes is often to improve the feed conversion rate, e.g. by reducing the viscosity or by reducing the anti-nutritional effect of certain feed compounds. Feed enzymes (such as phytase) can also be used, such as to reduce the amount of compounds which are harmful to the environment in the manure. When the enzyme granules of the present invention are to be used in food applications, the enzyme must be food qualitiy.

It is within the scope of the invention that at least one, preferably two, preferably 3 or more different enzymes are used. These can be enzymes from the same class, e.g. two different phytases or enzymes from different classes, e.g. a phytase and a xylanase.

In a preferred embodiment the enzyme is selected from the group consisting of of phytases, xylanases, phospholipases and glucose oxidases and mixtures thereof.

In one embodiment the enzyme does not include starch degrading enzymes (for example amylases). In some embodiments proteases may be excluded as these may cause harm if ingested.

In a preferred embodiment the enzyme is a phytase.

If the enzyme is a phosphatase, such as a phytase, then preferably the final granulate will have an activity of from 3,000 to 25,000, such as from 5,000 to 15,000, such as 5,000 to 10,000 such as from 6,000 to 8,000, FTU/g. If the enzyme is a plant cell wall degrading enzyme, for example a cellulase, and in particular a hemicellulose such as xylanase, then the final granulate may have an activity of the enzyme ranging from 3,000 to 100,000, preferably 5,000 to 80,000, and optimally 8,000 to 70,000. EXU/g. If the enzyme is a cellulase, such as β-gluconase, then the final granulate can have an enzyme activity of from 500 to 15,000, preferably from 1,000 to 10,000, and optimally from 1,500 to 7,000, BGU/g.

Phytase activity (FTU) is determined according to the procedure "ISL-method 61696" (manual vanadate assay). β-glucanase (BGU)activity is determined according to the procedure "ISL-method 62170" (manual viscosimetric assay). Endo-xylanase activity (EXU) is determined according to the procedure "ISL-method 62169" (manual viscosimetric assay). ISL-methods are obtainable on request from DSM N.V. Food Specialties, Agri Ingredients Group, Wateringseweg 1, P.O. Box 1, 2600 MA, Delft, The Netherlands.

The granules may comprise from 1 to 20, e.g. from 5 to 20, e.g. from 7 to 15 weight% of the enzyme(s). The enzyme(s) may be naturally occurring or recombinant.

A preferred process according to the invention therefore comprises:

a) mixing the (optional) water, enzyme, stabilizing agent and solid carrier comprising at least 15% (w/w) of an edible carbohydrate polymer, for example mixing the solid carrier and the at least one stabilizing agent with an aqueous enzyme-containing liquid;
b) optionally kneading the resulting mixture;
c) granulating, for example by mechanical processing, the mixture in order to obtain enzyme-containing granules, for example by using a granulator or by extrusion;
d) optionally spheronising the granules;
e) drying the resultant granules to obtain an enzyme-containing granulate.

In a preferred embodiment the enzyme-containing granulated obtained in step e) is further coated.

During the entire process one will aim to keep the maximum temperature to which the enzyme(s) are exposed to below 80°C.

A further aspect of the present invention relates to a process for the preparation of animal feed, or a premix or precursor to an animal feed, the process comprising mixing a granulate as defined in any of the claims 17 to 21 with one or more animal feed sub-

stances (e.g. seeds) or ingredients. This can then be sterilised, e.g. subjected to heat treatment. The resulting composition can then suitably be processed into pellets and optionally dried.

A further aspect of the present invention relates to a process for the preparation of a composition, or a premix or a precursor suitable for human nutrition, the process comprising mixing a granulate as defined in any of the claims 17 to 21 with one or more food substance(s) or ingredient(s). This can then be sterilised, e.g. subjected to heat treatment. The resulting composition can then suitably be processed into pellets and optionally dried.

Preferably the granules and feed or food substance(s) or ingredient(s) are mixed in a ration of granules: feed or food substance(s) or ingredients which is at lower than 1 g:1 kilo, preferably lower than 0,1 g : 1 kg.

A further aspect of the invention relates to the use of a granule as defined in any of claims 17 to 21 for human/and or animal nutrition. The invention therefore encompasses compositions such as animal feed compositions or compositions suitable for human nutrition. These compositions are preferably in the form of pellets (there may be 1-5, e.g. 2-4 dried granules per pellet). These composition can have a water content of from 10 to 20%, e.g. from 12-15%. The amount of enzyme(s) is suitably from 0.0005 to 0.0012%, such as at least 5 ppm.

A further aspect relates to a process for promoting the growth of an animal, the process comprising feeding an animal with a diet that comprises a granule as defined in any of claims 16 to 20.

Suitably the composition comprises from 0.05 to 2.0, such as 0.3 to 1.0, optimally 0.4 to 0.6 FTU/g of a phosphatase, e.g. a phytase. A xylanase may be present at from 0.5 to 50, e.g. 1 to 40 EXU/g. Alternatively or in addition a cellulase may be present at from 09.1 to 1.0, e.g. 0.2 to 0.4 BGU/g.

A further aspect of the present invention relates to the use of the granule as defined in any of claims 16 to 20 in, or as a component of, an animal feed or for use in an animal diet.

A further aspect of the invention relates to the use of at least one stabilizing agent selected from the group consisting of gummi arabicum and/or plant proteins in an enzyme containing composition to improve the pelleting stability of the enzyme.

Suitable animals include farm animals (pigs, poultry, livestock), non-ruminants or monogastric animals (pigs, fowl, poultry, marine animals such as fish), ruminants (bo-

vine or ovine, e.g. cows, sheep, goats, deer, calves, lambs). Poultry includes chickens, hens and turkeys.

Preferred features and characteristics of one aspect of the invention are equally applicable to another *mutatis mutandis*.

The following summarizes the invention.

1. A process for the preparation of enzyme-containing granule(s), the process comprising processing
   (i) at least one enzyme,
   (ii) a solid carrier which comprises at least 15% (w/w) of an edible carbohydrate polymer, and
   (iii) at least one stabilizing agent, wherein the stabilizing agent is selected from the group consisting of gummi arabicum and plant proteins and mixtures thereof.

2. A process according to claim 1 wherein water is added to the processing.

3. A process according to any preceding claim wherein the water and enzyme are provided as an enzyme-containing aqueous liquid.

4. A process according to claim 3 wherein the liquid is a filtrate derived from a fermentation process resulting in production of the enzyme.

5. A process according to any preceding claims wherein the granules are dried subsequent to the processing.

6. A process according to any preceding claim wherein the plant protein is selected from the group consisting of grain proteins, pulses proteins, vegetable proteins, and fruit proteins or hydrolysates thereof.

7. A process according to any preceding claim wherein the process comprises:
   a) mixing an aqueous liquid containing the enzyme with the solid carrier and the stabilizing agent;
   b) mechanically processing the mixture obtained in a) to obtain enzyme-containing granules; and
   c) drying the enzyme-containing granule(s) obtained in b).

8. A process according to any preceding claim wherein the processing is mechanical and comprises extrusion, pelleting, high-shear granulation, expansion, fluid bed agglomeration or a combination thereof.

9. A process according to any preceding claim wherein an enzyme-containing aqueous liquid, the solid carrier and the stabilizing agent are mixed and the resulting mixture kneaded before granulation.

10. A process according to any preceding claim wherein the processing is extrusion performed at low pressure and/or in a basket- or dome- extruder.

11. A process according to any preceding claim wherein the granule(s) are spheronised.

12. A process according to any preceding claims wherein the granule(s) are coated.

13. A process according to any preceding claim, wherein the enzyme is selected from the group consisting of phytases, xylanases, phospholipases and glucose oxidases.

14. A process according to claim 13 wherein the enzyme is a phytase.

15. A process according to any preceding claim, wherein if the enzyme is a phytase, the granule(s) will have phytase activity ranging from 4,000 to 20,000 FTU/g, preferably from 3,000 to 25,000 FTU/g and more preferably from 5,000 to 15,000 FTU/g.

16. Enzyme-containing granule(s) obtainable by a process as defined in any preceding claim.

17. Granule(s) comprising at least one enzyme, a solid carrier which comprises at least 15% (w/w) of an edible carbohydrate polymer, and at least one stabilizing agent, wherein the stabilizing agent is selected from the group consisting of gummi arabicum and plant proteins.

18. Granule(s) according to claim 17 which is coated.

19. Granule(s) according to claims 17 and/or 18, wherein the enzyme is selected from the group consisting of phytases, xylanases, phospholipases and glucose oxidases and mixtures thereof.

20. Granule(s) according to any of claims 17 to 19 wherein the enzyme is a phytase.

21. A process for the preparation of an animal feed, or a premix or precursor to an animal feed, the process comprising mixing granule(s) as defined in any of claims 16 to 20 with one or more animal feed substance(s) or ingredient(s).

22. A process for the preparation of a composition, or a premix or a precursor suitable for human nutrition, the process comprising mixing granule(s) as defined in any of claims 16 to 20 with one or more food substance(s) or ingredient(s).

23. A process according to any of claims 21 to 22 wherein the mixture of feed or food substance(s) and granule(s) is sterilised or treated with steam, pelletised and optionally dried.

24. Use of granule(s) as defined in any of claims 16 to 20 for human and/or animal nutrition.

25. A process for promoting the growth of an animal, the process comprising feeding an animal with a diet that comprises a granule as defined in any of claims 16 to 20.

Examples

All percentages are (w/w) unless otherwise specified.

EXAMPLE V 1 (Comparison)
Preparation of corn starch-based enzyme granules by kneading, extrusion, spheronisation and drying

In a first step corn starch was filled into a laboratory mixer (Loedige Typ M5 RMK) and slowly agitated. A mixture of an ultra-filtrate containing phytase, $ZnSO_4 \times 6\ H_2O$, PVA (polyvinyl alcohol) and additional water was poured into a beaker and dissolved/dispersed. This mixture was poured onto the corn starch in the Loedige mixer.

Thus, an enzyme containing dough was obtained by mixing and kneading 66% (w/w) of corn starch, 20% (w/w) of an ultra-filtrate containing phytase, 1% (w/w) of PVA, 0,3% (w/w) of $ZnSO_4 \times 6\ H_2O$ and 12,7% (w/w) of additional water at 100 – 250 rpm in the Loedige mixer.

In a second step this enzyme containing dough was extruded using an Fuji Paudal laboratory extruder, type DG-L1, to obtain a wet extrudate which was then rounded in a laboratory spheroniser to obtain round particles of an average diameter of 500 – 800 µm.

These particles were subsequently dried in a fluid bed dried (Niro-Aeromatic, type MP-1) for 60 - 90 minutes at a bed temperature of approximately 40°C. Approximately 1,5 kg of the wet granules were dried and subsequently cooled down. The obtained dry enzyme granules had an activity of 8920 FTU/g.

Example V 1	corn starch	Ultrafiltrate	PVA	$ZnSO_4 \times 6\ H_2O$	water
dough	66,0 %	20,0 %	1,0 %	0,3 %	12,7%
granules	83,6 %	8,6 %	1,4 %	0,4 %	6,0 %

EXAMPLE 1: soy protein as stabilizer

Enzyme (phytase) containing granules were obtained according to example V1.

A soyprotein (Supro 670; Name: IP Non GM; Batch IP 094/2003, distributed by Protein Technologies International, CAS No. 9010-10-0) was used instead of PVA.

The obtained dry enzyme granules had an activity of 7840 FTU/g.

Example 1	corn starch	ultrafiltrate	Soy protein	$ZnSO_4 \times 6\ H_2O$	water
dough	62,0 %	20,0 %	5,0 %	0,3 %	12,7%
granules	78,3 %	8,6 %	6,8 %	0,4 %	6,0 %

EXAMPLE 2 : soy protein as stabilizer

Enzyme (phytase) containing granules were obtained according to example V1.

Soyprotein Supro 1751 (Batch M 330000575 distributed by Protein Technologies International, CAS No. 9010-10-0), was used instead of PVA.

The obtained dry enzyme granules had an activity of 7890 FTU/g.

Example 2	corn starch	ultrafiltrate	soyprotein	$ZnSO_4 \times 6\ H_2O$	water
dough	53,5 %	18,7 %	9,4 %	0,3 %	18,1%
granules	71,7 %	8,5 %	13,4 %	0,4 %	6,0 %

EXAMPLE 3: potato protein as stabilizer

Enzyme (phytase) containing granules were obtained according to example V1.

A potato protein Alburex SP (SP, distributed by Roquette, CAS No. 100209-45-8) was used instead of PVA.

The obtained dry enzyme granules had an activity of 8110 FTU/g.

Example 3	corn starch	ultrafiltrate	potato protein	$ZnSO_4 \times 6\ H_2O$	water
dough	62,0 %	20,0 %	5,0 %	0,3 %	12,7%
granules	78,5 %	8,5 %	6,6 %	0,4 %	6,0 %

EXAMPLE 4: gum acacia as stabilizer
Enzyme (phytase) containing granules were obtained according to example V1.
Gum acacia (Instant Senegal, Spraygum IRX 51693, Batch 3S4120, distributed by Colloides Naturels International, CAS No. 9000-01-5) was used instead of PVA.

The obtained dry enzyme granules had an activity of 7720 FTU/g.

Example 4	corn starch	ultrafiltrate	gum acacia	$ZnSO_4 x$ 6 H_2O	water
dough	62,0 %	20,0 %	5,0 %	0,3 %	12,7%
granules	78,7 %	8,6 %	6,3 %	0,4 %	6,0 %

EXAMPLE 5: wheat protein as stabilizer
Enzyme (phytase) containing granules were obtained according to example V1.
A wheat protein Solpro 100 (Batch E 031579, distributed by Amylum CAS No. RN 100684-25-1) was used instead of PVA.

The obtained dry enzyme granules had an activity of 8570 FTU/g.

Example 5	corn starch	ultrafiltrate	wheat protein	$ZnSO_4 x$ 6 H_2O	water
dough	62,0 %	20,0 %	5,0 %	0,3 %	12,7%
granules	78,3 %	8,5 %	6,8 %	0,4 %	6,0 %

Comparison of pelleting stabilities
The different enzyme granules of the invention were subjected to a pelleting trial and their pelleting stability was compared to granules made according to the standard recipe (= Example V1 (ex. 2). The pelleting trial consisted of mixing the different enzyme granules with a standard broiler diet at a level of 1 g/kg feed. This enzyme containing feed was treated by injection of steam in a conditioner and immediately afterwards pelleted in a pelleting press to obtain feed pellets, which were subsequently cooled. Pellet temperature directly measured after pelleting press was 78°C. This type of process is typically used in feed industry to obtain feed pellets. All examples showed an increase of at least 10 %, some of over 20 % in pelleting stability as compared to example V1.

CLAIMS

1. A process for the preparation of enzyme-containing granule(s), the process comprising processing
   (i) at least one enzyme,
   (ii) a solid carrier which comprises at least 15% (w/w) of an edible carbohydrate polymer, and
   (iii) at least one stabilizing agent, wherein the stabilizing agent is selected from the group consisting of gummi arabicum and plant proteins and mixtures thereof.

2. A process according to claim 1 wherein the plant protein is selected from the group consisting of grain proteins, pulses proteins, vegetable proteins, and fruit proteins or hydrolysates thereof.

3. A process according to any preceding claim, wherein the enzyme is selected from the group consisting of phytases, xylanases, phospholipases and glucose oxidases

4. Enzyme-containing granule(s) obtainable by a process as defined in any preceding claim.

5. Granule(s) comprising at least one enzyme, a solid carrier which comprises at least 15% (w/w) of an edible carbohydrate polymer, and at least one stabilizing agent, wherein the stabilizing agent is selected from the group consisting of gummi arabicum and plant proteins.

6. A process for the preparation of an animal feed, or a premix or precursor to an animal feed, the process comprising mixing granule(s) as defined in any of claims 4 to 5 with one or more animal feed substance(s) or ingredient(s).

7. A process for the preparation of a composition, or a premix or a precursor suitable for human nutrition, the process comprising mixing granule(s) as defined in any of claims 4 to 5 with one or more food substance(s) or ingredient(s).

8. Use of granule(s) as defined in any of claims 4 to 5 for human and/or animal nutrition.

9. A process for promoting the growth of an animal, the process comprising feeding an animal with a diet that comprises a granule as defined in any of claims 4 to 5.

Abstract

A process for the preparation of an enzyme-containing granulate is disclosed where at least one enzyme, a solid carrier which comprises at least 15% (w/w) of an edible carbohydrate polymer, and at least one stabilizing agent, wherein the stabilizing agent is selected from the group consisting of gummi arabicum and plant proteins and mixtures thereof is mechanically processed into granules, and subsequently optionally dried. This enzyme granulate is suitable for the manufacture of animal feed compositions as well as for compositions for human nutrition. The compositions show improved enzyme stability during the pelleting process.

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Bemerkung TMR:Timer, ABR:Abruf, UKA:Umk.abr., 2IN:2in1TX, ORG:Origin.form.einst., BCH:BuchTx
FML:Frame lösch. Tx, MIX:Format-Mix, TX:Manuelle-Übertr, KRDS:KRDS, WLTG:Weiterltg
2.S.:Duplexseite, SP:Spezielle Originale
FCODE:Fcode, M-Box:Vertraul., BUL:Bulletin, RLY:Relais, RTX:Rel.-Tx, PC:PC-FAX
STOP: Stop Verbindung, Bes.: Besetzt, Fortf.: Fortfahren, K. Antw.: Keine Antwort
S.voll: Speicher voll, Hauptsch. aus: Hauptschalter Aus, Telefon: Empfang über Telefon

006 30.01.2004 16:21:20

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(Liste der diesem Antrag beigefügten Unterlagen) (Checklist of enclosed documents) (Liste des documents annexés à la présente requête)

Es wird hiermit der Empfang der unten bezeichneten Dokumente bescheinigt / Receipt of the documents indicated below is hereby acknowledged / Nous attestons le dépôt des documents ci-dessous

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Patente, Marken und Lizenzen GVX/A – C6
67056 Ludwigshafen
Bundesrepublik Deutschland

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Anmeldenummer / Application No. / N° de la demande		
Tag des Eingangs (Regel 24(2)) / Date of receipt (Rule 24(2)) / Date de réception (règle 24(2))	DREC	
Zeichen des Anmelders/Vertreters / Applicant's / Representative's ref. / Référence du demandeur ou du mandataire	AREF	0000056309/MST (EP-Erstanmeldung)
Nur nach Einreichung der Anmeldung bei einer nationalen Behörde: / Only after filing of the application with a national authority: / Seulement après le dépôt de la demande auprès d'un service national:		
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A.	Anmeldungsunterlagen und Prioritätsbeleg(e) / Application documents and priority document(s) / Pièces de la demande et document(s) de priorité	47			
1.	Beschreibung (ohne Sequenzprotokollteil) / Description (excluding sequence listing part) / Description (sauf partie réservée au listage des séquences)		1	28	
2.	Patentansprüche / Claim(s) / Revendication(s)		1	4	
3.	Zeichnung(en) / Drawing(s) / Dessin(s)	DRAW 1 #		/	
4.	Sequenzprotokollteil der Beschreibung / Sequence listing part of description / Partie de la description réservée au listage des séquences			/	
5.	Zusammenfassung / Abstract / Abrégé		1	1	
6.	[illegible]				
7.	Prioritätsbeleg(e) / Priority document(s) / Document(s) de priorité				
8.	[illegible]				
B.	[illegible]	48			
1.	[illegible]				
2.	Allgemeine Vollmacht / General authorisation / Pouvoir général				
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